PROTEOLYTIC CLEAVAGE OF SPARC RELEASES PEPTIDES THAT REGULATE ENDOTHELIAL CELL GROWTH AND ENHANCE ANGIOGENESIS IN VITRO

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The composition of the extracellular matrix is thought to modulate the initiation and extent of angiogenesis *in vivo* and is an important regulator of endothelial cell shape, gene expression, and proliferation *in vitro*. **SPARC** (osteonectin, BM-40) is a collagen-binding glycoprotein synthesized by endothelial cells responding to angiogenic stimulation and by vascular smooth muscle cells in response to injury. SPARC is also released from α-granules following platelet activation. Functional assays have demonstrated that SPARC inhibits adhesion and spreading of endothelial cells and fibroblasts. In a previous report, we identified sequences at both the N-terminus (SP_{5-23}) and C-terminus ($SP_{254-273}$) that also reversibly blocked endothelial spreading. We now show that proteolytic cleavage of SPARC occurred after the release of this protein from human platelets and that dibasic residues within the cysteine-rich region 2 of SPARC were sensitive to plasmin and to several other serine proteases. Comparison of cDNA sequences shows that amino acids 113-130, which contain several basic residues, are 100% conserved in frog, chicken, mouse, cow and man. A peptide corresponding to this sequence, $SP_{113-130}$, was used for studies on endothelial cells *in vitro*. Exposure of growth-arrested bovine aortic endothelial (BAE) cells to 0.1-10μM peptide resulted in a dose-dependent stimulation of cell proliferation; 10μM peptide caused a 50% increase in the incorporation of ^{3}H-thymidine. These levels of $SP_{113-130}$, did not appear to alter cell shape or the attachment of cells to tissue culture plastic. Addition of this peptide to angiogenic strains of BAE cells (strains which form cords and tubes spontaneously resulted in a does-dependent increase in the number of endothelial cords after 1-4 days of treatment. The effects were mimicked by smaller peptides that contained the sequence GHK from this region of SPARC and were potentiated by low levels of Cu^{++}. Proteolytic digests of SPARC, resolved by mass spectrometry, demonstrate the specific release of peptides that contain GHK. Previous work has shown that both SPARC and peptides that contain histidine (such as GHK) form complexes with Cu^{++}-ions under physiological conditions. These studies provide evidence that SPARC is proteolytically processed after secretion, that the peptides released are bioactive, and that the binding of Cu^{++} to SPARC or SPARC peptides could provide a previously unrecognized form of regulation for the activity of these proteins.

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